

# Watermelon

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## 1 Introduction

Watermelon (*Citrullus lanatus*) is a member of the cucurbit family (Cucurbitaceae). The crop is grown commercially in areas with long frost-free warm periods. Plants must be grown at a wide spacing because of their long, trailing vines. The exception is for dwarf cultivars where the plants can be grown at a tighter spacing. The crop may be established in the field by planting seeds or using containerized transplants. Management of plant pests (weeds, insects, and diseases, including nematodes) is essential during the production period. Three-fourths of the world production is grown in Asia, with China the leading country in production.

Watermelons are grown in most states of the United States, but the major producers are in the South and West (Florida, Georgia, California, and Texas) where the warm production season lasts longer. The fruit are harvested by hand, with the most experienced workers doing the cutting (removal of the fruit from the vine) and the others loading the bins or trucks. The fruit are shipped to markets throughout the United States, with some exported to Canada.

Watermelon fruit will keep for two to three weeks after harvest if they are stored properly at 10 to 15°C and 90% humidity. Besides whole watermelons, it is becoming popular to sell watermelon in pre-cut halves, quarters, slices, and chunks. Whole fruit usually are cut in the store under cold, aseptic conditions since the cut product does not ship or store well. Seedless watermelons are especially popular for pre-cut sales, since that shows their seedless quality.

In the 1800s, watermelon was grown mostly for local sales. However, with the development in the last few decades of rapid shipping in refrigerated railroad cars and trucks has led to distribution of watermelon throughout the United States from major production areas. Southern production areas begin shipping early in the year, and the harvest continues throughout the summer by moving to more northern areas.

Depending on the cultivar, watermelon fruit are produced in different sizes: ice box, small, medium, large, or giant; different shapes: round, oval, blocky, or elongate; different rind patterns: gray, narrow stripe, medium stripe, wide stripe, light solid, or dark solid; different flesh colours: white, yellow, orange, or red; and different types: seeded or seedless. Commercially, the most popular seeded cultivars are red flesh, blocky shape, and large sized (8–11 kg), like the cultivar Allsweet. For seedless watermelons, the popular cultivars are red flesh, oval shape, and medium sized (5–8 kg), like the cultivar Tri-X-313. Per capita consumption of watermelons in the United States is 7.2 kg.

Watermelon is served fresh as slices, as chunks (often in fruit salad), as juice, pickled rind, glacé candy, and as edible seeds (harvested from confectionary type cultivars). It is no longer just a summer fruit and is becoming an everyday fruit like apples, bananas, and oranges. The watermelon fruit is 93% water, with small amounts of protein, fat, minerals, and vitamins. In some arid regions, watermelon is used as a valuable source of water. The major nutritional components of the fruit are carbohydrates (6.4 g/100 g), vitamin A (590 IU), and lycopene (4,100 µg/100g, range 2,300–7,200), an anticarcinogenic compound found in red flesh watermelon. Lycopene may help reduce the risk of certain cancers, such as prostate, pancreas, and stomach. The lycopene content of the new dark red watermelon cultivars is higher than in tomato, pink grapefruit, or guava. Orange flesh types have only small amounts of lycopene, and the beta carotene content is similar to that of red flesh types. Canary yellow types do not contain lycopene, but do have a small amount of beta carotene. Watermelon seeds are rich in fat and protein.

Watermelon flowering and fruit development are promoted by high light intensity and high temperature. Watermelon is the only economically important cucurbit with pinnatifid (lobed) leaves; all of the other species have whole (non-lobed) leaves. The leaves are pinnately divided into three or four pairs of lobes, except for a non-lobed (sinuate) gene mutant controlled by the *nl* gene. Watermelon growth habit is a trailing vine. The stems are thin, hairy, angular, grooved, and have branched tendrils at each node. The stems are highly branched and up to 30 feet long, although there are dwarf types (*dw-1* and *dw-2* genes) with shorter, less-branched stems. Roots are extensive but shallow, with a taproot and many lateral roots.

Watermelon has small flowers that are less showy than those of other cucurbits. Flowering begins 4 to 8 weeks after seeding. Flowers of watermelon are staminate (male), perfect (hermaphroditic), or pistillate (female), usually borne in that order on the plant as it grows. Monoecious types are most common, but there are andromonoecious (staminate and perfect) types, mainly the older cultivars or accessions collected from the wild. The pistillate flowers have an inferior ovary, and the size and shape of the ovary is correlated with final fruit size and shape. In many cultivars, the pistillate or perfect flowers are borne at every seventh node, with staminate flowers at the intervening nodes. The flower ratio of typical watermelon cultivars is 7:1 staminate:pistillate, but the ratio ranges from 4:1 to 15:1.

The fruit of watermelon are round to cylindrical, up to 600 mm long and have a rind 10 to 40 mm thick. The edible part of the fruit is the endocarp (placenta). That contrasts with melon (*Cucumis melo*), where the edible part of the fruit is the mesocarp. Fruit as large as 120 kg have been recorded, but usually they weigh 4 to

16 kg. In Asia, even smaller watermelon fruit in the range of 1 to 4 kg are popular. That size is now becoming popular in the U.S. Fruit rind varies from thin to thick, and brittle to tough.

Seeds continue to mature as the fruit ripens and the rind lightens in colour. Seeds will be easier to extract from the fruit if the fruit is held in storage (in the shade or in a seed processing room) for a few days after removing them from the vine. If seeds are left too long in the fruit they will germinate *in situ*. There is no dormancy in watermelon seeds, so they can be harvested on one day, cleaned, dried, and planted on the next day. Seeds germinate in 2 days to 2 weeks depending on temperature and moisture conditions. Seeds will not germinate below 60°F. The optimum germination temperature is 85 to 90°F, especially for triploid seeds. For germination of triploid hybrid seeds, temperature and moisture are more critical, and it is especially important to avoid excess moisture.

## 2 Origin and Domestication

Watermelon has been cultivated in Africa and the Middle East for thousands of years, and in China since at least 900 AD. Watermelon was brought to the New World in the 1500s. In the United States, watermelon is a major vegetable crop that is grown primarily in the southern states. The major watermelon producing states are Florida, California, Texas, Georgia, and Arizona.

Through history, watermelon was distributed throughout the world as trade and knowledge of central Africa developed. The crop was grown in India by at least 800 AD, and in China by 1100 AD. The Moorish conquerors of Spain introduced watermelon into Europe, where it was noted in Cordoba in 961 AD and Seville in 1158 AD. The spread of watermelon into northern Europe was relatively slow, and it was not noted in the British Isles until late in the 16th century, perhaps because of the generally unfavourable climate for watermelon culture in much of Europe. About this time, watermelons were introduced into the New World, with culture of the plants noted in the Massachusetts colony in 1629. The introduction of watermelon into other parts of the world has followed established trade routes.

Watermelon has been improved by domestication and formal plant breeding from a late maturing vine with small fruit having hard, white flesh and bland or bitter taste, into an early maturing, more compact plant with large fruit having edible, sweet flesh. In the last century, plant breeders working in public or private programs in the United States and around the world have released cultivars having disease resistance, dwarf vines, larger fruit, higher sugar content, higher lycopene content, seedlessness, and new flesh colours, such as scarlet red, dark orange, and canary yellow. Recent advances in the breeding of seedless triploid hybrids have resulted in renewed popularity of watermelons, and per capita consumption has increased 37% since 1980.

## 2.1 Centres of Origin

Watermelon is thought to have originated in southern Africa because it is found growing wild throughout the area, and reaches maximum diversity there. It has been cultivated in Africa for over 4,000 years. The citron (*Citrullus lanatus* var. *citroides*) grows wild there, and is thought to be related to the wild ancestor of watermelon. In 1857, David Livingstone reported watermelon growing profusely after unusually heavy rainfall in the Kalahari Desert (the current nations of Namibia and Botswana). The natives there knew of sweet as well as bitter forms growing throughout southern Africa. De Candolle, in 1882, considered the evidence sufficient to prove that watermelon was indigenous to tropical Africa, more specifically the southern parts of Africa.

*Citrullus colocynthis* is considered to be a wild ancestor of watermelon, and is now found native in north and west Africa. Fruit of colocynth are small, with a maximum diameter of 75 mm. The flesh is bitter and the seeds are small and brown. Crosses of *C. lanatus* with *C. colocynthis* produced F<sub>1</sub> hybrids with nearly regular meiosis. The pollen was 30 to 40% fertile, and 35% of the seeds were fertile. The original wild watermelons probably had hard, non-sweet, sometimes bitter, white flesh, similar to the citron and colocynth.

## 2.2 Centres of Diversity

The primary centre of diversity for watermelon is southern Africa, with wild relatives also found in west Africa. The secondary centre is China, and related species can be found in India. Areas of the middle east as well as countries near the Mediterranean Sea may also be good places to collect old land races and wild accessions of *Citrullus*.

T. W. Whitaker considered *Citrullus colocynthis* to be the likely ancestor of watermelon. It is morphologically similar to *C. lanatus*, but with bitter fruit and small seeds. However, the bitter forms of *C. lanatus* were considered the probable ancestor of watermelon by others. That theory was supported based on the fact that they had the same number of chromosomes as *C. lanatus*, were freely intercrossable, and were found in the same areas of Africa and Asia. Citron was considered to be an intermediate stage between the primitive, bitter form of *C. lanatus* and the cultivated form of today.

Although *Citrullus* species grow wild in southern and central Africa, *C. colocynthis* also grows wild in India. India and China may be considered secondary centres of diversity for the genus. Cultivation of watermelon began in ancient Egypt and India, and is thought to have spread from those countries through the Mediterranean area, Near East, and Asia. The crop has been grown in the United States since 1629.

Germplasm is the foundation of breeding programs, so germplasm collection and evaluation are important aspects of breeding. Priorities for collection of *Citrullus* germplasm include India, especially the Indo-Gangetic plains and areas in the northwest parts of the country; Africa including the south and southwest (Kalahari Region); southern areas of the former USSR and Iran; and tropical Africa.

Recent work in germplasm collection and exchange has provided the USDA germplasm system with a total of 51 *Citrullus* accessions that were collected during a scientist exchange visit with the People's Republic of China led by Wehner in 1993. Later, in 1996, a team of four researchers led by Wehner collected germplasm of *Citrullus* in the Republic of South Africa.

### 3 Varietal Groups

Watermelon (*Citrullus lanatus*) has 22 chromosomes ( $2n=22$ ,  $x=11$ ). The genus *Citrullus* belongs to the subtribe Benincasinae. In 1930, L.H. Bailey proposed dividing cultivated watermelon *C. vulgaris*, into botanical variety *lanatus* and botanical variety *citroides*. The genus *Citrullus* has been studied taxonomically, and recently has been divided into four species: *C. lanatus* (syn. *C. vulgaris*), *C. ecirrhosus*, *C. colocynthis*, and *C. rehmi*. *C. ecirrhosus* is more closely related to *C. lanatus* than either is to *C. colocynthis*. There are two other closely related species: *Praecitrullus fistulosus* from India and Pakistan, and *Acanthosicyos naudinianus* from southern Africa. Other members of the Cucurbitaceae with 22 chromosomes include *Gymnopetalum*, *Lagenaria*, *Momordica*, *Trichosanthes*, and *Melothria*. None appear to be closely related to watermelon.

#### 3.1 Citron

Watermelon has a close relative, citron or preserving melon, which is *C. lanatus* var. *citroides*. Its rind is used to make pickles, and the fruit are fed to livestock. The flesh of the citron is white or green, and may vary from bland to bitter tasting. Citron grows wild in the United States where it causes problems as a weed in crop production areas of the south, especially in Florida, Georgia, and Texas. Watermelon seed production fields should be isolated from weedy areas of citron since these two botanical varieties cross readily.

#### 3.2 Egusi

Some watermelon accessions in the USDA-ARS germplasm collection show a particular phenotype usually described by breeders as Egusi seed type. These accessions have been misclassified on occasion. The Egusi watermelon is commonly known in Nigeria and the Congo as wild watermelon, Egusi melon, or Ibara. The Egusi watermelon is widely cultivated in Nigeria, where the protein- and carbohydrate-rich seeds are used as a regular part of the diet. The fruit are not edible because of their bitter, hard, white flesh. The origin of the Egusi phenotype is uncertain, and the developmental genetics of this seed phenotype are not known. Its seeds are coated by an adherent layer of tissues that may be remnants of nucellar tissues. The tissues are visible only after the second to third week of seed development, and can be removed at maturity for commercial use of the seeds. Egusi type watermelons are used to feed cattle in Africa. Egusi has sometimes been confused with *Citrullus colocynthis* and as a result, the Egusi watermelon has been

sometimes considered a common name for *Citrullus colocynthis*. *Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus* [= *Colocynthis citrullus* L.] is the cultivated watermelon, and can have Egusi phenotype, but accessions of the Egusi type are not colocynths (*Citrullus colocynthis*).

## 4 Genetic Resources

### 4.1 Germplasm Repositories

Several germplasm collections, along with current cultivars marketed by seed companies, represent the major sources of germplasm for watermelon breeders interested in the United States market. The USDA collection is stored at the Regional Plant Introduction Station, Griffin, Georgia with the backup collection at the National Seed Storage Laboratory, Fort Collins, Colorado. There are 1644 accessions in the collection, with about 90% currently available to researchers, and the rest needing to be regenerated to increase seed quantity or germination percentage. The collection includes representatives of all *Citrullus* species and botanical varieties. In addition, approximately 300 heirloom cultivars are kept at the National Seed Storage Laboratory.

The Cucurbit Genetics Cooperative has curators who volunteer to collect and maintain seeds of gene mutants published for many of the cultivated cucurbit species. Some gene mutants are no longer available, but small amounts of seeds of some of the gene mutants can be obtained from the curators for that species, T. C. Wehner and S. R. King.

Additional collections are kept by seed savers and other groups interested in heirloom cultivars, and by watermelon breeders around the United States. There are also watermelon germplasm collections in other countries that are being kept for use by the local research community.

### 4.2 Important cultivars

Watermelon cultivars have been described in the vegetable cultivar lists maintained by the American Society for Horticultural Science. A complete set of descriptions for all vegetable crops, including watermelon, from lists 1 through 27 are available on the world wide web. Seeds are available for many of the open pollinated and inbred cultivars on the list, but a large number of cultivars that are no longer available. Watermelon breeders should obtain and evaluate a sample of the cultivars available to become familiar with the diversity of germplasm. It is also useful to observe the improvement in horticultural traits that has been made in cultivars developed over time.

A breeding program usually is started by intercrossing the best cultivars currently available, or by crossing the best cultivars with accessions having one or more useful traits missing from the elite cultivars. Thus, in the beginning a watermelon breeder will need to obtain seeds of the best cultivars, a set of cultivars developed at different

times in the past, a set of accessions from germplasm repositories, and lines with useful or interesting gene mutants.

A survey of popular cultivars in the ten major watermelon-producing states in the United States by D.N. Maynard in 2000 indicated that popular cultivars for commercial production were almost all hybrids, with few open-pollinated cultivars being used commercially. Popular diploid (seeded) open-pollinated cultivars ('Allsweet', 'Black Diamond', 'Calsweet', 'Crimson Sweet', 'Jubilee II', and 'Legacy') were grown mostly in one state each, suggesting regional adaptation or local demand. Hybrids generally were grown in several states, suggesting they have wider adaptation. The 'Allsweet' type, generally considered to be of high quality, was represented by more than half of the listed cultivars (three of the open-pollinated and 11 of the hybrids). The most popular diploid (seeded) cultivars were 'Sangria' and 'Royal Sweet' (seven states), 'Fiesta' (six states), and 'Mardi Gras' and 'Regency' (five states). For triploid (seedless) cultivars, almost half of the cultivars were 'Tri-X-313' type. The most popular triploid cultivars were 'Tri-X-313' (ten states), 'Summer Sweet 5244' (nine states), 'Millionaire' (eight states), 'Genesis' (five states), and 'Tri-X-Shadow' (four states).

In order to develop improved cultivars for an industry in a particular region of the world, the watermelon breeder will need to have seeds of cultivars, breeding lines, populations, plant introduction accessions, and gene mutants that express the traits of interest at a high level. The breeder should identify a source that has the highest level of expression. That would be true whether the trait is quantitatively inherited (fruit yield, earliness, size, sweetness) or qualitatively inherited (dwarfness, anthracnose resistance, flesh colour). If there is a choice of accession for a particular trait (for example, white flesh), it is better to use an adapted accession with the best genetic background. Thus, 'Cream of Saskatchewan' would be a better choice to use in the development of white flesh cultivars for use in the United States, than a wild-type, white-fleshed citron having large vines, late maturity, hard flesh, bitter flavour, large green seeds, and seed dormancy.

There were no defined cultivars of watermelon before the 1820s. Early cultivars include 'Black Spanish' (imported to United States from Portugal in 1827), 'Carolina' (available at least since 1827), and 'Imperial', 'Mountain Sprout', 'Seminole', and 'Mountain Sweet' (introduced by southern growers from 1840 to 1850). Other heirloom cultivars include 'Bradford', 'Clarendon', 'Odell', 'Ravenscroft', and 'Souter' (originating in South Carolina before 1850). Classic watermelon cultivars include 'Peerless' or 'Ice Cream' (1860), 'Phinney Early' (1870), and 'Georgia Rattlesnake' developed by M.W. Johnson in Atlanta, Georgia about 1870.

Planned cultivar development programs began in the United States in 1880 to 1900. Important cultivars developed for the southern United States included 'Cuban Queen' developed and marketed by Burpee in 1881, 'Round Light Icing' (1885), 'Kolb Gem' developed by Reuben Kolb of Alabama in 1885 and marketed by D.M. Ferry, 'Florida Favourite' selected from the cross of 'Pierson' x 'Georgia Rattlesnake' by Girardeau in Monticello, Florida in 1887, 'Dark Icing' developed in 1888 by D.M. Ferry, and 'Dixie' selected from the cross of 'Kolb Gem' x 'Cuban Queen' or 'Mountain Sweet' by George Collins in North Carolina and marketed by Johnson and Stokes. Important cultivars developed for the western United States included

'Chilean' (black or white seeded) brought from the west coast of South America and introduced to California in 1900, 'Angeleno' developed by Johnson and Musser in Los Angeles, California in 1908, and 'Klondike Solid' and 'Klondike Striped' of unknown origin developed about 1900. Important cultivars developed for shipping include 'Tom Watson' developed by Alexander Seed Co. in Augusta, Georgia in 1906, and 'Stone Mountain' developed by Hastings Co. in Atlanta, Georgia in 1924.

Important cultivars developed in the latter part of last century have built on past accomplishments. 'Charleston Gray' (USDA, Charleston, 1954), 'Crimson Sweet' (Kansas State University, 1963), 'Calhoun Gray' (Louisiana State University, 1965), and 'Dixielee' (1979), 'Jubilee' (1963), and 'Smokylee' (1971) (all from the University of Florida) have high resistance to Fusarium wilt. 'Dixielee' (University of Florida, 1979) and 'Sangria' F<sub>1</sub> (Syngenta - Rogers Brand, 1985) have dark red flesh. 'Millionaire' F<sub>1</sub> 3x (Harris Moran, 1992) and 'Royal Jubilee' F<sub>1</sub> (Seminis) have consistently high yields. 'Crimson Sweet' (Kansas State University, 1963) and 'Sugarlee' (University of Florida, 1981) have high soluble solids. 'Kengarden' (University of Kentucky, 1975) has dwarf vines. 'Tri-X-313' F<sub>1</sub> 3x (Syngenta - American Seedless, 1962) is seedless. 'Minilee' (University of Florida, 1986), 'Mickylee' (University of Florida, 1986), 'New Hampshire Midget' (University of New Hampshire, 1951), 'Sugar Baby' (M. Hardin, Oklahoma, 1955), and 'Tiger Baby' (Seminis) are icebox size. 'Yellow Doll' (Seminis, 1977) has canary yellow flesh.

## 5 Major Breeding Achievements

### 5.1 Qualitative Traits

The inheritance of watermelon traits has been studied extensively, and single genes have been identified that are of value to plant breeding programs. Examples include *A* for monoecious vs. andromonoecious sex expression, *Ar-1* and *Ar-2* for resistance to anthracnose races 1 and 2, *C* for canary yellow flesh colour, *dw-1* and *dw-2* for dwarf vines, *E* for non-explosive rind, *F* for non-furrowed fruit surface, *Fo-1* for Fusarium wilt resistance, *g<sup>s</sup>* for striped green rind pattern, *Go* for non-golden rind at maturity, *M* for non-mottled fruit skin, *o* for oval rather than elongate fruit shape, *Pm* for resistance to powdery mildew, *s* and *l* for short seeds, *Scr* for scarlet red flesh, *y<sup>o</sup>* for orange flesh, and *Y* for coral red flesh.

A non-lobed leaf is a mutant expressed beginning in the seedling stage that is controlled by a single recessive gene. The single-gene trait can be useful for indication of hybrid plants. Hybrid seeds can be produced on one inbred line used as the female parent and having non-lobed leaves. If it is pollinated using bee pollination in an isolation block, and the male parent has normal, lobed leaves, then it will be possible to distinguish hybrid from non-hybrid at the seedling stage in the commercial seed lot. The hybrid seeds can then be planted in excess in grower fields and the non-lobed seedlings (produced by self- or sib-pollination) can be removed to leave just hybrid plants. Alternatively, non-hybrid seedlings can be removed from the flats during transplant production.



## 5.2 Inbreeding Depression and Heterosis

Watermelon is monoecious, and is naturally cross-pollinated like maize. However, there is not as much inbreeding depression or heterosis as one might expect. This is similar to other cucurbits such as cucumber (*Cucumis sativus*) and melon (*Cucumis melo*). It has been suggested that the lack of inbreeding depression is due to the small population size used by farmers during the domestication of the species. Watermelon plants are large, so only a few plants probably were grown in each area. Therefore, even with monoecious sex expression and insect-pollinated flowers, there would have been considerable inbreeding among the few plants representing the population. Since there is little inbreeding depression in watermelon, inbred lines are developed using self-pollination with little loss of vigour from the parental population.

In studies of heterosis in watermelon, some estimates have shown a 10% advantage of the hybrid over the high parent, but only for some parental combinations. The small amount of heterosis observed in watermelon hybrids makes hybrids unnecessary for high yielding commercial cultivars since inbreds should perform as well. However, hybrid cultivars are useful for combining traits inherited in a dominant fashion from the two parents. Examples of such traits include red or canary yellow flesh, resistance to Fusarium wilt and anthracnose, and resistance (actually lack of susceptibility) to powdery mildew. Hybrids also permit the protection of proprietary inbred lines from unauthorized use. However, one of the most important uses of hybrids is the production of seedless cultivars. The primary method for production of seedless watermelons involves the cross of a tetraploid female parent with a diploid male parent to produce a triploid, which will be sterile, and therefore, seedless. Currently, triploid hybrids are the most practical method for production of seedless watermelons.

## 6 Current Goals of Breeding

In watermelon breeding, it is important to have proper expression for many traits. Furthermore, lack of one key trait (such as scarlet red flesh colour) can make the cultivar unattractive for use in a particular market. This is the common situation for breeding most horticultural crops. With so many traits to work on, it is difficult to make improvements. However, by starting with a leading cultivar, and making crosses with other elite cultivars, it is possible to maintain the expression level for most traits while making gains for one or two traits. Important traits for watermelon are described below.

### 6.1 Vines

Vine length of watermelon varies from dwarf to long. For example, ‘Charleston Gray’ and ‘Jubilee’, large-fruited cultivars, have vines up to 30 feet long. Short or medium length vines are well suited to cultivars with small or medium sized fruit. For example, ‘Sugar Baby’, ‘New Hampshire Midget’, and ‘Petite Sweet’ are short vined, and ‘Crimson Sweet’ has intermediate vine length.

Dwarf mutants have been discovered in watermelon. Two genes cause dwarfing when they are in homozygous recessive condition: *dw-1* and *dw-2*. 'Kengarden' has the genotype *dw-1 dw-1*. Another gene mutant (Japanese Dwarf, *dw-2 dw-2*) has increased branching from the crown. Dwarf plants having both sets of genes (*dw-1 dw-1* and *dw-2 dw-2*) have hypocotyls 50% the length of normal vining plants, so can be selected in the seedling stage.

## 6.2 Sex Expression

Most current cultivars are monoecious, and that appears to be the preferred type of sex expression for commercial seed production of inbred lines and hybrid cultivars. Andromonoecy (*aa*) is recessive to monoecy.

Most cultivars have a ratio of 7 staminate to 1 perfect or pistillate flower. There are some cultivars with a ratio of 4 staminate to 1 pistillate flower. It may be possible to breed for gynoeceious sex expression by selecting for increased proportion of pistillate nodes in a segregating population. There is no advantage to andromonoecious sex expression, since the perfect flowers must be pollinated by bees in order to set fruit. Thus, they are no more likely to set without bees or to be self-pollinated, than monoecious cultivars.

Male sterility is useful for the production of hybrid seeds without the requirement for expensive hand pollination. The glabrous male sterile (*gms*) mutant provides male sterility, but the plants are less vigorous, have poor seed set, and are susceptible to cucumber beetles because they lack hairs. A second male sterile mutant, the Chinese male sterile (*cms*), has been more useful for hybrid production.

Fruit can be set parthenocarpically. Although there are no gene mutants that make plants parthenocarpic, fruit set may be achieved without pollination by applying growth regulators to the plants. Thus, commercial production of seedless watermelon may be possible in areas where bees have been excluded by applying growth regulators at a particular growth stage to diploid pistillate flowers that would otherwise produce seeded fruit.

## 6.3 Yield

Yield varies among watermelon accessions and current cultivars. Growers want high weight per acre of marketable size fruit, with a low percentage of culls. The yield goal expressed by many growers is at least one load (45,000 lb.) per acre.

In the production of triploid hybrids, up to one third of the field must be planted to a diploid seeded cultivar. Therefore, higher yield of seedless watermelon per acre could be obtained by using a more efficient pollenizer that would allow more than two thirds of the field to be planted to the triploid cultivar. Alternatively, parthenocarpic fruit set (genetic or hormone-induced) to stimulate fruit set would permit the entire field to be planted to the triploid cultivar.

## 6.4 Earliness

Early maturity is desirable because prices for watermelon usually are best at the beginning of the local season. However, late maturity is associated with cultivars that have large fruit size and high yield. Thus, it may be necessary to sacrifice some earliness to obtain high yield or large fruit. Time from pollination to fruit harvest ranges from 26 days for early maturing, small-fruited cultivars such as ‘Petite Sweet’ to 45 days for large-fruited cultivars such as ‘Super Sweet’.

The selection process for early maturity should involve both days from seeding or transplanting to first fruit set, and days from first fruit set to fruit harvest. Days to fruit harvest should be based on fruit having fully developed sugars as verified by a hand-held refractometer or by taste evaluation.

## 6.5 Fruit Type

Fruit size is an important consideration in a breeding program since there are different market requirements for particular groups of shippers and consumers. The general categories are: mini (<4.0 kg), icebox (4.0-5.5 kg), small, sometimes called pee-wee (5.5-8.0 kg), medium (8.0-11 kg), large (11-14.5 kg), and giant (>14.5 kg). Fruit size is inherited in polygenic fashion, with an estimated 25 genes involved. Shippers in the United States work with particular weight categories, such as 8.0-11 kg for seeded and 6.5-8.0 kg for seedless.

Old cultivars tend to have larger fruit size than current cultivars, because one of the things growers were interested in was winning competitions for fruit weight. Competitions are still being held to grow the largest fruit, but commercial production concentrates on high quality. Another reason for larger fruit in the past is that they are more efficient for hand harvest and shipping; large fruit handled individually permit more weight to be moved per unit. Also, there was demand for large fruit to be sold or served by the slice for restaurants and cafeterias. Today, most supermarkets request seedless fruit that weigh 6.5-8.0 kg for standard and 2.0-4.0 kg for mini types.

Small- or medium-fruited types were the result of adapting watermelon to the northern areas of the United States. Cultivars developed for the northern United States were bred from early maturing Asian cultivars brought from Japan and Russia. A. F. Yeager produced the early cultivars ‘White Mountain’ and ‘New Hampshire Midget’ from sources, which have 1.0-2.0 kg fruit with a 65-day maturity. The early cultivar ‘Petite Sweet’ has 2.0-4.5 kg fruit.

Even though icebox cultivars with 4.0-5.5 kg fruit have been developed to fit easily in a small refrigerator, most of the demand in the marketplace for small fruit has been met using sections cut from a large fruit. A large watermelon fruit cut into quarters has the same weight as an icebox melon, but it has a different shape, and consumers can see what they are buying. ‘Sugar Baby’, a small-fruited cultivar popular in some parts of the world, was selected in Oklahoma by M. Hardin in 1956.

Fruit shape is also an important part of market type. The general categories are round, oval, blocky, or elongate. There is one gene involved in round vs. elongate, with the  $F_1$  being intermediate (blocky). In some cases, fruit shape is related to

cotyledon shape at the seedling stage. Plants with elongate fruit have elongate cotyledons, and plants with round fruit have round cotyledons. However, others have concluded that selection for fruit shape at the seedling stage is ineffective. Among old cultivars with elongate-shaped fruit, there was greater susceptibility to production of gourd-neck or bottle-neck fruit, which are culls. Old cultivars with round fruit were more susceptible to hollowheart. Thus, some of the first hybrids were made between elongate and round inbreds to reduce the incidence of these defects. Recently, genetic resistance to those defects has been incorporated into new cultivars, and has made fruit shape less important to consider.

The third area of importance in market type is rind pattern, which can be gray, striped, or solid. Stripes on the rind can be narrow, medium, or wide where the stripes are the dark green areas. The striped pattern can be on light green or medium green background. Solid rind colour can be light or dark green. Solid dark green is dominant to gray rind pattern. Solid dark green is dominant to striped, and striped is dominant to solid light green rind pattern. However, the striped pattern can be seen on a solid dark green fruit after the colour has been bleached by the sun. The stripes can be over a light or medium green background. For example, 'Dixielee' has narrow stripes on a light green background, whereas 'Florida Favourite' has narrow stripes on a medium green background.

In addition to the common rind patterns, there is furrowed vs. smooth rind, controlled by the recessive gene, *f*. Most current cultivars have smooth rind. Another interesting mutant is golden rind, which is controlled by the recessive gene, *go*. Its usefulness as an indicator of fruit ripeness is limited because the change in fruit colour at fruit maturity is accompanied by chlorosis of the leaves. Furthermore, it does not appear to be a reliable indicator of ripeness, and may be disadvantageous for yield, especially if the grower is using a multiple harvest system.

## 6.6 External Fruit Quality

Rind durability is important on cultivars that are to be shipped to market. On large-fruited cultivars, the rind should be thick and tough; whereas on small-fruited cultivars, the rind should be thin and tough. Rind thickness should be a small percentage of flesh diameter to keep it in a balanced proportion for best appearance. Large-fruited cultivars look better with a thicker rind, and need the extra protection for postharvest handling and shipping. The rind can be tough and hard as in 'Peacock' or tough and soft as in 'Calhoun Gray'. Brittle rind as in 'New Hampshire Midget' is not useful for cultivars that are to be shipped to market.

Rind flexibility can be tested by cutting a 1/16 to 1/8 inch x 3 inch piece of rind from a fruit and bending the rind into an arc. If the rind bends into a tight arc, it is flexible and tough. If it breaks early in the attempt, it is tender and explosive.

Rind toughness can be measured by driving a spring-loaded punch into the rind. A tough rind would require more force to punch through, whereas a tender or brittle rind requires less force. Watermelon breeders often use faster methods to test for rind toughness, however. One method is to drop the fruit onto the ground from a particular height (for example, knee height) to see whether it breaks open or not. The drop height would depend on the soil type of the field being used. Another method is

the “thumb” test, where the breeder presses on the rind at a particular location on each fruit. If the rind breaks when only a small amount of force is applied, then it has a tender rind; otherwise it should be resistant to shipping damage.

## 6.7 Internal Fruit Quality

Flesh colour is one of the primary traits consumers look for in a watermelon fruit. Colour can be scarlet red, coral red, orange, canary yellow, salmon yellow (golden), or white. Coral red (*YY*) is dominant to orange ( $y^Oy^O$ ), which is dominant to salmon yellow (*yy*). Canary yellow (*CC*) is dominant to non-canary yellow (*cc*), and epistatic to (overcomes) the *y* locus for red-orange-salmon yellow. Coral red is recessive to the white flesh colour, which is found in citron. Scarlet red colour (*Scr*) from ‘Peacock’ has been used to develop many new cultivars because of its attractive colour. Cultivars with dark red flesh include ‘Dixielee’, ‘AU-Sweet Scarlet’, ‘Red-N-Sweet’, and ‘Sangria’.

Sugar content is a major component of flavour. Breeders select for high sugar content as indicated by taste and refractometer readings. Refractometer readings are easily made in the field using a handheld unit, and provide data on percentage of soluble solids (°Brix). These translate to sugar content, which should be a minimum of 10%. Newer cultivars have Brix as high as 14%. Some cultivars have higher levels of fructose, which tastes sweeter than sucrose. The difference in taste is not measured by a refractometer.

Selection should be made for good watermelon flavour, independent of sweetness (sugar content). Flavour should include freedom from bitterness, which is controlled by a single dominant gene, and may be introduced in crosses with *C. colocynthis* accessions. Another component is caramel flavour as in ‘Sugar Baby’ fruit, which some taste testers find unpleasant. The flavour is sometimes associated with dark red flesh colour. Its inheritance is not known, but caramel flavour does respond to selection. Thus, breeders should select lines with mild (not bitter) taste, high sugar content (°Brix), freedom from caramel flavour, and excellent “watermelon” taste. It is important that cultivars with excellent taste be included as checks in all selection blocks to provide a comparison for the plant breeder. Examples of cultivars with good quality that are commonly used include ‘Allsweet’, ‘Crimson Sweet’, and ‘Sweet Princess’.

Flesh texture is an important part of internal quality. Watermelon fruit can have flesh that is soft or firm, and fibrous or crisp. The objectives for plant breeders should be to develop cultivars with flesh that is firm and crisp. The genes controlling those traits are not known, but they are heritable.

## 6.8 Watermelon Rind Pickles

The use of watermelon to make rind pickles is a small but interesting segment of the home gardening sector. Homeowners and small industries use the leftover watermelon crop, especially from cultivars having thick and crisp rind, to produce pickles. The watermelon fruit consists of the exocarp, mesocarp, and endocarp. The endocarp is the seed-containing part that is consumed as food, and the mesocarp and

exocarp are usually referred to as the rind. The rind is used for making pickles after removing the thin exocarp, leaving the crisp, white mesocarp.

Many obsolete cultivars were discontinued from use in the market because of their thick rind, so they would be obvious candidates for use in making watermelon pickles. Some of those old cultivars are still used by home gardeners and heirloom collectors, and seeds are available from seed companies. ‘Tom Watson’, ‘Georgia Rattlesnake’, and ‘Black Diamond’ are three heirloom cultivars with good flavour, attractive rind pattern and colour, and thick rind. In addition, many hybrids currently cultivated for fruit production by commercial growers have rind that is thick enough for pickle production. Cubes of 10 mm per side can be cut from most of the hybrids tested, thus allowing the pickling of the rind of many modern cultivars, including seedless watermelons.

## 6.9 Seeds and Seedlessness

Seed colour can be white, tan, brown, black, red, green, or mottled. White seed colour usually is not preferred since it suggests that the fruit is immature, and can make it difficult to distinguish mature from immature seeds. On the other hand, white seeds may be a useful objective for the development of near-seedless cultivars that have few, small, and inconspicuous seeds. Black seed colour is attractive with scarlet red or canary yellow flesh colour. Black, brown, or tan seeds look good with orange flesh colour.

Seed size should be large for confectionery (edible seeded) type, and small or medium sized for the standard (edible flesh) type. A new seed size mutant discovered recently is called tomato seed. The seed size is about half that of the small watermelon seed size, and is controlled by a single recessive gene, *ts*.

Seed number should be high for the confectionery type, but should be low or medium for the edible flesh type. Seed number should be lower in small-fruited cultivars so that the seeds will not appear to include more than the usual percentage of the fruit volume. Seed number should be high enough to make seed production economical, but low enough to make the flesh easy to eat.

In theory, seedless triploid hybrids should provide higher yield than diploid hybrids because no energy is used in seed production. However, in practice this may not be the case. Fruit production in triploids is limited by the availability of viable pollen to induce fruit set.

During the development of tetraploid inbreds, seed yield is often low in early generations, so selection for fertility is essential. Some tetraploids are more fertile than others, and should be selected to keep seed costs low for triploid hybrid production, since the hybrid seeds are produced on the tetraploid parent line.

Triploid hybrids are generally seedless, but occasionally hard seed coats form in the fruit. The presence of objectionable seed coats is affected by environment, but can also be selected against in the development of the inbred parents of the hybrid. Inbred parents that do not develop objectionable seed coats in the fruit in different production environments should be selected for triploid hybrids.

## 6.10 Seedling Disease Tests

Disease resistance is an important objective of most breeding programs. Screening for resistance to several important diseases using greenhouse seedling tests is useful, and provides several advantages. Plants that are found to be resistant to the diseases being tested can be transplanted from the test flats to soil or other growth medium in bags or pots where they can be grown and self-pollinated, or crossed with other lines. Greenhouse tests can be run at a time when plants cannot be grown outside, permitting more generations of testing each year, and the disease testing greenhouses can be isolated from other watermelon research to keep the diseases from spreading. For seedling tests on gummy stem blight tests, plants should be isolated in one greenhouse, virus tests in another greenhouse, and breeding work in another greenhouse to prevent diseases from spreading from one to the other.

For some diseases such as anthracnose, it is useful to have a humidity chamber to incubate the disease after inoculation. A humidity chamber can be built on a greenhouse bench, usually one humidifier for each 3 m<sup>2</sup> of bench space. An air conditioner can be used to keep the temperature cool, since some diseases do best in cool and humid conditions. The greenhouse temperature is usually kept between 21° and 32°F for optimum plant growth, and the humidity chamber is usually kept between 47° and 57°C for optimum disease development. A less expensive option for disease chambers is to build a frame on a greenhouse bench and cover it with polyethylene film on the top and sides. Humidifiers placed inside the chamber several hours before disease inoculation should be able to raise the relative humidity above 95%.

## 6.11 Fusarium Wilt Resistance

Fusarium wilt is caused by *Fusarium oxysporum* f. sp. *niveum*. The disease was first reported in 1889 in Mississippi, and was widespread throughout the southern parts of the United States by 1900. Three types of pathogen spores are commonly observed: small, colourless, oval, non septate microconidia; large, sickle shaped, septate macroconidia; and thick walled circular chlamydospores. There are three races known: 0, 1, and 2. Most current cultivars are resistant to race 0, and some also are resistant to race 1. Race 2 was discovered more recently, and occurs mainly in the south central production areas such as Texas and Oklahoma, but it also has been found in Florida.

Race 0 causes wilt in older, susceptible cultivars such as ‘Florida Giant’, ‘Black Diamond’, and ‘Sugar Baby’. Race 1 is more virulent than race 0 and affects more plants within susceptible cultivars, but does not affect resistant ‘Calhoun Gray’. Race 2 is highly virulent and can affect otherwise resistant cultivars such as ‘Calhoun Gray’, ‘Summit’, ‘Smokylee’, and ‘Charleston Gray’. Races of Fusarium can be identified using differentials. ‘Sugar Baby’ and ‘Black Diamond’ are susceptible to all the three races; ‘Quetzali’, ‘Mickylee’, ‘Charleston Gray’, and ‘Crimson Sweet’ are susceptible to races 1 and 2, while ‘Calhoun Gray’ is susceptible to only race 2. Resistance to race 2 is available in PI 296341 and PI 271769 (Table 1).

**Table 1.** Reaction of cultivars or accessions of watermelon to *Fusarium* wilt races 0, 1 and 2 (S=susceptible, R=resistant).

<b>Fusarium wilt race</b>			<b>Cultivar or accession</b>
<b>0</b>	<b>1</b>	<b>2</b>	
S	S	S	Black Diamond (or Sugar Baby)
R	S	S	Quetzali (or Mickylee)
R	M	S	Charleston Gray (or Crimson Sweet)
R	R	S	Calhoun Gray
R	R	R	PI 296341 (or PI 271769)

*Fusarium* can survive in soil as a saprophyte. The pathogen is spread locally by moving soil, compost, manure, water, tools, and machinery from one field to another, as well as by humans and animals moving between fields. The pathogen can also persist on infested seeds for more than 2 years.

*Fusarium* enters plants through root tips and openings in roots where lateral roots emerge. Presence of root-knot nematodes is also thought to increase the incidence of the disease. After penetration, the fungus grows into the xylem where it accumulates materials that plug the xylem and cause wilting. Watermelon is attacked at all growth stages by the pathogen. At the seedling stage there is damping-off, and cotyledon wilt results in slower growth and stunting. The vascular tissue inside wilted stems may be discoloured. A white or pink coloured fungus growth usually appears on the surface of dead stems in wet weather conditions. The ideal temperature for infection and disease development is 80°F. However, seedling rot occurs at soil temperatures of 61° to 65°F, while seedling wilt is severe between 77° to 82°F. The disease is also promoted by high soil organic matter.

The first *Fusarium* wilt resistant cultivar ‘Conqueror’ was released in 1908. It was developed by W.A. Orton of the USDA using a wilt-resistant citron accession crossed with ‘Eden’. ‘Conqueror’ did not have high fruit quality, so was not grown much after its release. However, cultivars developed using resistance from ‘Conqueror’ such as ‘Iowa Belle’ and ‘Iowa King’ had improved fruit quality, so were used commercially. More recent cultivars such as ‘Calhoun Gray’, ‘Smokylee’, and ‘Dixielee’ have resistance, as well as improved horticultural performance.

Two types of *Fusarium* wilt resistance are known, having different patterns of inheritance. Resistance to race 1 in ‘Calhoun Gray’ is controlled by a single dominant gene, with some modifier genes, and provides a high level of resistance that is easy to transfer into new breeding lines. There is also a source of resistance to race 1 which is controlled by several recessive genes. That source of resistance has been difficult to fix at a high level in stable, inbred lines. Cultivars resistant at high inoculum levels are ‘Dixielee’ and ‘Smokylee’. In wild species, resistance to *Fusarium* has been reported to be polygenic. Resistance to race 2 has been reported in PI 296341, and the selection PI 296341-*FR* is resistant to all three races of *Fusarium*. Also, PI 271769 was reported to be highly resistant to race 2.



## 6.12 Anthracnose Resistance

Anthracnose caused by *Colletotrichum lagenarium* is an important disease of watermelon in the United States. Symptoms caused by this pathogen may occur on leaves, stems, and fruit. Lesions on leaves are irregular shaped, limited by the leaf vein, and brown to black in colour. Lesions on the stem are oval shaped and tan coloured with a brown margin. Lesions similar to those found on stems and leaves also appear on the fruit. Older fruit show small water-soaked lesions with greasy, yellowish centres that are somewhat elevated.

Seven races of the anthracnose pathogen have been reported. Races 4, 5, and 6 are virulent in watermelon, but races 1 and 3 are most important. Many cultivars are resistant to races 1 and 3, and resistance to race 2 will be needed in the near future.

The first source of resistance to anthracnose was identified in an accession, Africa 8, sent to D.V. Layton of the USDA by R.F. Wagner in Umtali, South Africa. Layton developed anthracnose resistant ‘Congo’, ‘Fairfax’, and ‘Charleston Gray’ from that source. Resistance was later found to be inherited as a single dominant gene, *Ar-1*. The gene provides resistance to races 1 and 3, but not to race 2. ‘Crimson Sweet’ and many other current cultivars have that source of resistance. Several genes were found to be responsible for resistance to Race 2.

PI 189225, PI 271775, PI 299379, and PI 271778 have been reported to carry resistance to complex *Colletotrichum* species. Some of the other sources of resistance to anthracnose reported in the literature are PI 203551, PI 270550, PI 326515, PI 271775, PI 271779, and PI 203551. ‘R 143’ was reported to be resistant to race 2 of the pathogen. PI 512385 had the highest resistance to race 2 of the pathogen from a screening test involving 76 plant introductions.

## 6.13 Gummy Stem Blight Resistance

Watermelon is one of the most susceptible of the cucurbit species to gummy stem blight, caused by *Didymella bryoniae*. The disease occurs throughout the southern United States, particularly the southeast. Field and greenhouse tests are available, but the results are variable, and it can be difficult to get reproducible results.

The USDA collection of plant introduction accessions has been screened for gummy stem blight resistance by several teams of researchers. Some accessions have resistance to the disease, including PI 189225 and PI 271778.

## 6.14 Powdery Mildew Resistance

Watermelon is one of the most resistant cucurbit species to powdery mildew (*Sphaerotheca fuliginea*). However, there are a few regions of the world where powdery mildew is a problem on watermelon. For example, watermelons grown in southern India are affected with the disease, but not in northern India. In southern India, ‘Arka Manik’ is resistant to powdery mildew. The *pm* gene causes susceptibility to the disease, but most cultivars have the resistance allele. Powdery mildew is becoming more of a problem in the United States, especially in the western states, and has been reported in the southeastern states as well.

### 6.15 Yellow Vine Resistance

Yellow vine is a relatively new disease of watermelon, caused by an unknown, phloem-limited bacterium. Evidence indicates that leafhoppers vector the disease. The disease was first observed in central Texas and Oklahoma in 1991 and has caused severe losses in early-planted watermelon in some years. In 1998, the disease was detected in watermelon and pumpkin in Tennessee. Production areas of Georgia, Florida, and other parts of southeastern United States may be at risk in the future. Low levels of resistance or tolerance have been identified in a few open-pollinated and hybrid cultivars, although the mechanism of resistance is unknown. Research is needed to identify good sources of resistance.

### 6.16 Bacterial Fruit Blotch Resistance

Bacterial fruit blotch of watermelon is a serious disease of seedlings and fruit caused by *Acidovorax avenae* subsp. *citrulli*. Disease incidence increases under high humidity or where overhead irrigation is used. The disease was first reported to occur in commercial watermelon production areas in the United States in 1989. Early-season outbreaks can result in total loss of fruit by harvest time. Bacterial fruit blotch is also reported to attack cantaloupe fruit in the field, as well as other cucurbits. Bacterial fruit blotch epidemics during 1994 in certain states in the United States resulted in litigation, and had a devastating effect on the watermelon industry. Currently, most seed companies require growers to sign waiver forms to reduce the possibility of litigation. Some companies have restricted seed sales in certain states where the risk of disease is high. Seed costs have increased due to the changes in the seed handling, packaging and testing required for reducing the incidence of disease.

The characteristic symptoms of bacterial fruit blotch are the appearance of a dark olive green stain, or blotch, on the upper surface of infected fruit. Apart from attacking the fruit, the pathogen is also reported to attack the leaves and seedlings, and can be seed transmitted. D.L. Hopkins and co-workers reported that fermentation of seeds for 24 to 48 hours followed by 1% hydrochloric acid or 1% calcium hypochlorite treatment for 15 minutes prior to washing and drying were the most effective treatments for bacterial contaminated watermelon seeds. This treatment is for diploids; triploid seed germination is drastically reduced by fermentation. However, an effective, cost efficient, and environmentally safe method for disease control would be development of resistant cultivars.

A seedling test for early screening of watermelon fruit blotch was developed in 1992, and research on a few watermelon lines using this test has been reported. There has been some research to identify genetic resistance in the watermelon germplasm collection. Based on seedling tests, PI 295843 and PI 299378 were reported to be resistant to the pathogen. In 1993, D.L. Hopkins and co-workers conducted a study of 22 cultivars and 2 PI accessions for resistance to fruit blotch of watermelon and reported that none were immune to the pathogen. Research is underway to find sources of resistance in the germplasm collection.

Fruit resistance to the pathogen appears to be related to rind colour and ploidy, with diploid cultivars having light rind colour being most susceptible and triploid

cultivars with dark rind colour being less susceptible. Fruit with stripes appeared to be intermediate in their resistance. Detached leaf tests have been developed that are effective in screening plants for resistance in a breeding program.

### **6.17 Bacterial Rind Necrosis Resistance**

Bacterial rind necrosis is caused by *Erwinia* species. However, some other bacterial species (*Pseudomonas*, *Enterobacter*, and *Bacillus*) are also known to cause similar symptoms. Typical symptoms of bacterial rind necrosis on watermelon fruit are characterized by a light brown, dry, hard area of discoloration interspersed with light areas generally limited to the rind. The disease was first reported in Texas in 1968. The most resistant cultivars in studies conducted in Florida over a 3-year period were ‘Sweet Princess’ and ‘Jubilee’, while the most susceptible were ‘Klondike Blue Ribbon’ and ‘Louisiana Queen’.

### **6.18 Root-Knot Nematode Resistance**

Watermelon is susceptible to root-knot nematodes caused by *Meloidogyne* spp. The USDA collection of plant introduction accessions is being screened for resistance. Root-knot resistance may be an important future breeding objective if resistant accessions are identified.

### **6.19 Virus Resistance**

The main virus problems in watermelon production in the United States are papaya ringspot virus-watermelon strain (PRSV-W, formerly watermelon mosaic virus-1), watermelon mosaic virus-2 (WMV-2), and zucchini yellow mosaic virus (ZYMV). The watermelon germplasm collection has been screened for resistance to some virus diseases. Accessions reported to be resistant to WMV-2 are PI 244018 and PI 244019. Resistance to ZYMV is found in PI 482299, PI 482261, PI 595203, and PI 255137. Research is in progress to identify sources of resistance to PRSV-W as well. Multiple virus resistance will be an important breeding objective for new cultivars in a few years.

### **6.20 Other Disease Resistance**

Verticillium wilt is an increasing problem in the western United States, but little is known about sources of resistance. Resistance to *Alternaria* leaf spot has been identified in cultivars such as ‘Sugar Baby’, ‘Fairfax’, and ‘Calhoun Gray’.

### **6.21 Physiological Disease Resistance**

Many of the watermelon fruit defects have a genetic component. Breeders should select lines to be free of defects under conditions conducive to the problem. Fruit defects include hollowheart, rind necrosis, blossom-end rot, and cross stitch. Hollowheart is a separation of the tissue within the endocarp caused by rapid fruit

growth and weak tissue. More research is needed to identify sources of defect resistance, and environmental conditions that help reduce their frequency.

## 6.22 Insect Resistance

Little research has been done on insect resistance in watermelon. This may be due to the fact that most insect pests can be controlled with insecticides. The major arthropod (insect and arachnid) pests of watermelon are aphids, pickleworm, spider mite, and spotted, striped, and banded cucumber beetles.

PI 299563 is resistant to melon aphid (*Aphis gossypii*). ‘Congo’ and ‘Giza 1’ were the most resistant of five accessions evaluated for resistance to spider mite. Several genes were found to control non-preference type resistance to spotted cucumber beetle in ‘Hawkesbury’ x a resistant accession. Resistance to spotted and banded cucumber beetles was due a single recessive gene.

A single dominant gene, *Fwr*, was responsible for resistance to the melon fruit fly (*Dacus cucurbitae*) in the watermelon line J18-1. ‘Afghan’ is reported to have resistance to red pumpkin beetle (*Aulacophora foveicollis*), and ‘Blue Ribbon’ and ‘Crimson Sweet’ are resistant to pickleworm.

## 6.23 Stress Resistance

Little research has been done on stress resistance in watermelon. Water stress is an important cause of reduced yield in watermelon. It may be that some genotypes are more efficient in water use than others, but it probably will be difficult to develop highly efficient cultivars since watermelon fruit have very high water content. In Israel, deep-rooted cultivars are used in unirrigated desert areas.

Pollination problems are responsible for improper fruit development. It is necessary for all three lobes of the stigma to be fully pollinated if the fruit is to develop fully, and without curvature. Proper fruit development requires adequate numbers of honeybees or bumblebees during flowering, along with weather that is conducive to pollination. Bumblebees can be more effective pollinators than honeybees. Cold, rainy weather leads to poor pollen shed, and hot weather often leads to reduced bee activity. In the case of triploid hybrids, it is necessary to have up to one third of the field planted to a diploid pollenizer to assure adequate fruit development in the triploids which are male sterile.

Growers plant early in the season, often using transplants and plastic mulch (with row covers in some cases) when there is a danger of frost. Cucurbits are susceptible to chilling injury at air temperatures below 42°F. Chilling injury is a concern in watermelon because of the value of early harvested fruit. There might be chilling resistance in the watermelon germplasm collection that could be incorporated into new cultivars as has been done in other cucurbits. Watermelon appears to be more chilling resistant than melon and cucumber. Symptoms of chilling are white areas on the cotyledons and white or light brown margins on the fully expanded leaves. Chilling injury is increased by a longer duration of chilling, lower temperature, high intensity of light during chilling, high wind speed during chilling, or a higher growth temperature before chilling occurs. Watermelon is thermophilic, meaning that plants

have a high optimum growth temperature. Although the optimum is probably 80-90°F, temperatures above 90°F reduce growth rate, and can reduce fruit yield. Above 105°F, plants can be injured, and young leaves will be light green with yellow margins.

Measles is a condition where green-brown spots develop on the fruit surface, covering a small area or even the entire surface, and starting out as minute water-soaked areas. The spots become tan, slightly raised areas with necrotic centres. The symptoms occur when excessive guttation is encouraged by periods of high humidity or during the early fall production season when the humidity is high and the nights are cool. The fruit symptoms become evident 21-25 days after the conducive environmental conditions occur. There is usually no economic loss from the stress, and it might be controlled by reducing the amount of irrigation in the fall production season.

## 7 Breeding Methods and Techniques

Major objectives for watermelon breeding include proper fruit type, early maturity, high fruit yield, high sugar content, tough flexible rind, and proper seed type. It is important to determine breeding objectives carefully before starting cultivar development. For example, seed type changes significantly for different market classes. Parental lines for seedless hybrids should have small seeds, whereas confectionery seed types should have large seeds. For commercial cultivars, black seeds are preferred because of their contrast with red, yellow, or orange flesh. Also, white seeds indicate immaturity to buyers, so white mature seed colour can be a confusing trait for them. Most of the old cultivars are diploid, open-pollinated or inbred lines, but hybrid diploid and hybrid triploid cultivars are taking over the commercial market in the United States.

After determining the breeding objectives, methods for measurement of the traits of interest should be developed, selection methods should be determined (specifying the operations to be carried out for each generation), and parents with high expression of the traits of interest should be chosen. Vine type should be long for commercial production and dwarf (bush) for home garden. It may also be possible to use the dwarf plant type for once-over harvest in commercial production. Sex expression should be monoecious, with a ratio of 7 staminate:1 pistillate flowers, or better (preferably 4:1). Andromonoecious sex expression and ratios of 15:1 are more typical of older cultivars.

For production in most areas of the United States, watermelon must have resistance to *Fusarium* wilt. Races 0 and 1 are common, and race 2 is becoming important, especially in Texas and Oklahoma where plastic mulch culture and fumigation are less common. Production areas in the southern United States usually have anthracnose race 1 and may also have problems with race 2. Gummy stem blight is a disease for which resistance is needed in most southern production areas. Powdery mildew is becoming a problem, especially in the western United States (possibly because of a new race), and should be a breeding objective for new cultivars. Bacterial fruit blotch was a problem in the 1990s, and resistant accessions

have been identified. The disease can be effectively controlled by genetic resistance and by large-scale seed testing followed by destruction of contaminated seed lots. Protection from viruses in the United States production areas should include resistance to papaya ringspot virus-watermelon strain (formerly watermelon mosaic virus-1), watermelon mosaic virus (formerly watermelon mosaic virus-2), and zucchini yellow mosaic virus.

Finally, breeding objectives should emphasize early maturity, high fruit yield, durability for shipping, high internal quality, freedom from internal defects (hollowheart and rind necrosis), and proper seed type in a diploid (seeded) or triploid (seedless) hybrid. Internal quality traits include dark red flesh, high sugar content, proper sugar to acid ratio, excellent flavour, high nutritional value (vitamins and lycopene), firm (not soft) and non-fibrous texture. Seeds should be black colour, medium size (or small for inbreds to be made into tetraploids), and few to medium quantity per fruit (few for consumers, but medium to keep seed costs down). Flesh colour should be dark red (*Y* gene with modifier genes) with uniform colour throughout the fruit. For specialty types, flesh colour could be bright orange (*y<sup>o</sup>* gene), canary yellow (*C* gene), or white (*Wf* gene). Other colours such as salmon yellow (*y* gene) exist, but are not preferred because the flesh looks overmature. Older cultivars have light red flesh, but dark red is becoming the preferred type. Diploid inbreds should be made into tetraploid inbreds and tested for fertility, seed yield, and ability to set fruit using controlled pollination. Tetraploid lines for use in triploid seedless hybrid production can be induced with colchicine. Finally, triploid hybrids should be tested for absence of seed coats in the fruit within a range of production environments.

## 7.1 Pollination Methods

Watermelon is a cross-pollinated species with monoecious or andromonoecious flowering habit. There is a popular myth that watermelon should not be grown close to other cucurbits such as cucumber, cantaloupe, or squash because of an adverse effect on horticultural traits such as flavour. However, watermelon will not cross with any other cucurbits except for species within the genus *Citrullus*. Furthermore, there is no effect of foreign pollen on fruit development (xenia) in watermelon.

## 7.2 Greenhouse Pollinations

Controlled pollinations can be made easily in a greenhouse or screenhouse since that eliminates the need to cover individual flowers the previous afternoon to protect them from pollinating insects such as bees. The greenhouse or screenhouse should be well sealed to prevent insects from getting in. In those structures, pollinations should be made in the morning, and plant maintenance work should be left for the afternoon. Computer controlled heating and cooling, and automated irrigation and fertilization make it possible to operate the greenhouse with fewer labour inputs.

Greenhouse plants can be grown in ground beds, plastic bags or pots containing the growth medium, or in various liquid media such as ebb and flow benches or nutrient film technique. If pots or bags are used, different container sizes should be

evaluated to obtain the proper plant size. A good pot size for proper growth of watermelon plants is 200 mm diameter. Plants grown in larger pots will have longer vines that are more difficult to train and prune, larger fruit, and more seeds per pollination.

In the greenhouse, plants are usually trained vertically onto supports such as strings held by overhead wires. This saves floor space and makes better use of available light. The overhead wire should be 2 m above the walkway to permit most workers to reach the trellis without standing on a ladder, while being able to walk under it without ducking. Plants should be pruned to one main stem, usually with no branches. Because of their weight, fruit must be supported in a sling. Stem length of most watermelons usually requires that plants be trained up the string to the trellis wire, and back down again. Plants should be given sufficient floor space in the greenhouse to grow and flower. For elite cultivars and breeding lines, each plant should have 0.18 m<sup>2</sup> or more. It may be necessary to give wild accessions more space, perhaps 0.36 m<sup>2</sup> per plant or more.

In some latitudes, it may be necessary to provide supplemental lighting for plant growth. We find it difficult to grow plants in Raleigh, North Carolina in the winter without extra lighting. However, plants grow well and produce flowers, fruit, and seeds properly when grown in the spring (February through June) and fall (July through November) seasons.

### 7.3 Field Pollinations

Natural pollination of watermelons in the field is usually by honeybees that visit the flower to collect pollen and nectar. Bumblebees also are effective pollinators. Hand pollination of watermelon flowers is usually less effective than bee pollination. It is necessary to protect flowers from bee visits before and after making controlled pollinations. Flowers open shortly after sunrise and remain open for 1 day. Usually a pistillate flower and the staminate flower below it (proximal to it) open on the same day, making self pollination possible. Many breeders have found that hand pollination is more effective between 6 and 9 am than later in the day.

The two main methods for protecting controlled pollinations from insect pollination in the field are to begin pollinating before bees become active in the morning, or to cover the flowers the previous afternoon. For the first method, pollinations can be made on newly-opened flowers, which are then covered to keep bees away. This method requires less time per pollination, but care must be taken to stop pollinating when bees are observed in the field. Staminate and pistillate flowers can be covered with gelatin capsules (size OO), cotton wool, plastics caps, or paper rolled into a cylinder (often, holding a pencil inside as the paper is rolled) and closed at one end by folding. It is also possible to use inverted styrofoam or plastic cups (6-12 oz. size) held over the flower (and onto the soil surface) with a J-shaped wire (about 10 gauge thickness) stuck through the cup, or by a wooden stake glued to the cup. Breeders have also made flower covers using mesh or cloth bags, which in some cases are supported by a wire frame that can be stuck into the ground over the flowers to be protected.

The second method requires that flowers predicted to open the next morning be capped the previous afternoon. These flowers will be one or two nodes above the flowers (toward the shoot apex) that are newly opened, and should have some yellow colour in the petals. Flowers more than three nodes above the newly opened ones that are completely green will probably not open the next day. Capping of flowers is most useful if done on sunny days, since the pollen does not shed freely after rainy or cloudy days. The following morning, the caps are removed, flowers pollinated, and the caps replaced to keep bees away. This method permits the pollination crew to keep working longer as bees begin to work the field.

In a large field pollination nursery, workers often prefer to mark the flowers that have been capped in the afternoon with a flag (for example, white), which is then exchanged with a flag of a different colour (for example, blue) after the pollination has been made. Thus, it is easy to go to the white flags in the morning to make the pollinations, and to go to the blue flags in the afternoon to check whether the pollinations from previous mornings are developing properly. The setting of one fruit inhibits other fruit on the same plant from setting, so it is useful to remove pistillate flowers that have not been used for controlled pollinations as the pollinating crew moves through the field in the afternoon.

Andromonoecious plants have perfect flowers as well as staminate ones. Unfortunately, perfect flowers will not set fruit without being hand pollinated, or visited by a pollinating insect, so they are no more likely to be self-pollinated than pistillate flowers. After pollinating a pistillate flower, a tag is placed on the peduncle or on the stem just below the peduncle. Placing the tag on the stem causes less damage to the pollinated flower and developing fruit. The tag usually has the plot number of the female and male parents and the date the pollination was made. It can also have the initials of the person making the pollination, and the name of the study involved.

Controlled pollinations are made by removing a recently opened staminate flower from the plant to be used as the male parent. The petals of the staminate flower are bent back until they break. The flower can then be used like a paintbrush to pollinate a recently-opened pistillate flower on the plant to be used as the female parent.

A nursery for field pollination should be designed to make it easy to make controlled pollinations, and care for the plants. Direct seeding or transplants can be used. For direct seeding, the seeds should be treated with a registered fungicide before planting. Use of herbicides will significantly reduce the need for hand weeding. For transplants, plastic mulch and drip irrigation will help with weed control. Drip irrigation, or other low-level system (furrow, sub-irrigation) is superior to overhead irrigation to keep the plants dry, so hand pollinations can be made without having to wait for the watering to be completed, and to avoid having pollination caps washed off the flowers.

Pollinations are made easier by planting the lines to be crossed together in one area. Lines to be self-pollinated can be planted together in a second area. It is useful to plant each pair of lines to be crossed in adjacent rows or tiers.

If it is difficult to make self-pollinations in the field on a particular set of lines (perhaps selections from a trial), one or more cuttings can be taken from each of the plants to be selected. The cuttings can be rooted in moist sand in a greenhouse by



burying the bottom (proximal) internode, with two to five nodes of leaves above. The resulting plants can be transplanted from the rooting bench to the greenhouse for trellising and self- or cross-pollination of the selections to produce seeds for the next generation.

## 7.4 Breeding Plans

Once the breeder has determined the objectives of the program, the choice of parental materials is one of the most important aspects of a breeding program. Using knowledge of the crop and predicting the traits consumers will be interested in having in future cultivars, the breeder gathers parental lines for crossing. The breeder should know which parent will contribute the traits of interest, and which methods will be used to evaluate the progeny for those traits. Thus, it is often necessary to collect and evaluate large numbers of PI accessions, cultivars, and breeding lines for the traits of interest to identify appropriate parents to use in the program. This work often continues in parallel with the main part of the breeding program.

The next step is to determine the breeding method to use for each part of the program. It is important for the breeder to consider the advantages and disadvantages of particular breeding methods, and how they can be incorporated into the overall breeding plan. Also, it is common to use more than one breeding method at a time in order to accomplish several sets of objectives. For example, one part of the program might be to use recurrent selection to develop a base population with general adaptation and the proper fruit type that also has high yield and early maturity. A second part of the program might be to use pedigree selection on the cross of two lines to develop inbred lines with the high yield, early maturity, and proper fruit type of one parent, and the dark red flesh colour, high sugar content, and firm crisp flesh texture of the other parent. A third part of the program might be to use backcross breeding to make a canary yellow flesh version of an elite red-fleshed hybrid with top performance.

## 7.5 Recurrent Selection

Although watermelon is a cross-pollinated crop, population improvement methods popular in some cross-pollinated crops have not been used. The main reason for that appears to be the large size of the plants, and the low rate of natural outcrossing that occurs. Also, because there are few plant breeders working on watermelon, and because of the requirement for many qualitative traits to be present in the new cultivars being tested for release, it is expensive to spend additional years in population improvement for quantitative traits.

It may be possible to improve quantitative traits such as yield in watermelon using recurrent selection i.e. repeated selection and massing of selected plants, but the populations should probably be developed initially to have the necessary qualitative genes in them. Those would include proper flesh colour, fruit size, and disease resistance. Due to large plant size and a 5-month generation time, recurrent selection methods should be those that have few generations per cycle, and few plants per family (or single-plant selection).

One approach would be to develop an elite population by intercrossing two to four of the best red fleshed hybrids available, trying to choose a set that was genetically unrelated. A population with a wide genetic base could also be developed by intercrossing 20 or more elite cultivars by hand for two or more generations, and using bees in an isolation block for two or more generations before beginning a mild selection pressure for important quantitative traits such as yield. Simple recurrent selection could be used for selection among single-plant hills for a set of highly heritable traits. A more complex method such as reciprocal recurrent selection would permit simultaneous improvement of two populations for combining ability for yield. This would be an expensive program to run, but would produce two populations that could be used to develop inbreds to be used as the female and male parents (respectively) of elite hybrids.

During population development, it would be necessary to identify methods for yield testing that were efficient for use in large yield trials. The usual guidelines for recurrent selection are to test at least 200 individuals (or progenies of individuals) per population, and to select at least 20 to intercross for the next cycle of selection. A yield trial involving 200 replicated families would require more resources than many breeding programs could afford if the trial were done using current methods.

Recurrent selection could be used to improve quantitative traits, such as yield, which are difficult to improve using qualitative methods such as pedigree and backcross breeding. Each year, the improved population would be used to begin the development of inbred lines to feed into other parts of the breeding program.

## 7.6 Pedigree Breeding

Probably the most common method for watermelon breeding is pedigree. In pedigree breeding, the breeder begins by choosing two or more adapted parents, which complement each other in their traits. For example, one parent might be generally good (yield, earliness, type) except for disease resistance and the other might be generally good (yield, earliness, type) except for fruit quality. The objective would be to produce new lines with high yield, early maturity, proper type, high fruit quality, and good disease resistance. The cultivars or breeding lines are crossed to form the hybrid ( $F_1$ ) generation, which is then self- or sib-pollinated to form a segregating ( $F_2$ ) population. The  $F_2$  is self- or sib-pollinated while selecting for traits having high heritability to form the  $F_3$  generation. If multiple plants are tested from each selected  $F_2$  plant, then the breeder concentrates on selecting the best plants in each of the best  $F_3$  families. This might include selection in the seedling stage in the greenhouse in the  $F_2$  and  $F_3$  generations for disease resistance such as Fusarium wilt races 0, 1, and 2 and anthracnose races 1 and 2.

Beginning at the  $F_4$  generation, selection would begin to emphasize family-row performance for quantitative traits. Plants within family-rows that have excellent performance for qualitative traits should be selected for the next generation. As the families reach six generations of self-pollination ( $S_6$  or  $F_5$ ), they become more uniform, and can then be handled as inbred lines. This could include selection using eight-plant plots for early flowering, number of pistillate flowers, and fruit number.

The number handled might decrease from 54  $F_2$  plants of a cross to 36  $F_3$  families, 24  $F_4$  families, and 18  $F_5$  lines.

Single-seed-descent is a modification of pedigree breeding in which inbred lines are developed rapidly by self-pollination in greenhouses and winter nurseries, and selection is not practiced until later generations, such as  $S_3$  to  $S_6$ . This method requires less record keeping and works better where the main objective is to improve quantitative traits such as yield and earliness, rather than qualitative traits such as flesh colour and disease resistance. However, traditional pedigree breeding is probably the more useful method for watermelon since there are many qualitative traits that can be selected in early generations. In that way, plants or families having unsuitable traits that are simply inherited (such as poor fruit flesh colour) can be eliminated in early generations. Otherwise, they would be carried along until the  $S_3$  to  $S_6$  generation when field-testing would be practiced in the single-seed-descent breeding method.

### 7.7 Backcross Breeding

Backcross breeding is used to transfer one qualitative (highly-heritable) trait into an otherwise superior inbred. The superior inbred is referred to as the recurrent parent. Often, six generations of selection and backcrossing to the recurrent parent are used to recover the genotype of the recurrent parent (except for the addition of the new trait) without the other undesirable traits from the non-recurrent (donor) parent. Two versions of the backcross method are used depending on whether the gene of interest is recessive or dominant.

For the transfer of a trait controlled by a recessive gene, the recurrent parent is crossed with the donor parent, and the  $F_1$  backcrossed to the recurrent parent. In one scheme, the  $F_1$  is self-pollinated to produce the  $F_2$ , which will segregate for the trait of interest. Individuals having the trait can then be backcrossed to the recurrent parent to produce the  $BC_1$ . The  $BC_1$  generation is then tested for the trait, and individuals having it are self-pollinated once again to produce a segregating generation for selection and backcrossing to the recurrent parent. The process is repeated until the  $BC_6$  generation when the best individuals are self-pollinated and selected for the trait to produce the improved inbred. The inbred does not need to be tested extensively in trials, because it will be identical to the original inbred, but with one new trait.

For the transfer of a trait controlled by a dominant gene, the recurrent parent is crossed with the donor parent, and the  $F_1$  backcrossed to the recurrent parent. The  $BC_1$  generation is then tested for the trait, and individuals having it are backcrossed to the recurrent parent. The process is repeated until the  $BC_6$  generation when the best individuals are self-pollinated and selected for homozygous expression of the trait using progeny testing.

### 7.8 Inbred Development

The best selections from the recurrent selection program should be self-pollinated each cycle to begin inbred development. Pedigree selection, and backcross breeding

result in the production of elite inbred lines. Each year, those inbred lines that are produced from the different parts of the breeding program should be increased by self-pollination, tested for useful horticultural traits, and used in the production of tetraploid inbred lines, as well as directly for the production of diploid hybrids based on the traits they have, and what is needed by the market.

Isolation blocks or screen cages can be used to make large seed increases of the inbreds if that is needed. Isolation blocks should be away from other watermelon fields, requiring a separation of at least 1 mile. Bees should be provided in the isolation block or cage by bringing in one strong hive, unless there are sufficient numbers of wild bees.

## 7.9 Hybrid Testing

The final stage of breeding is to produce hybrids for testing. Hybrids are usually made between two monoecious inbreds. For triploid hybrid production, the seed parent should have a distinctive rind pattern that has recessive inheritance. For hybrid production with less labour input, the seed parent could be male sterile. The seed increase of the male sterile inbred would be accomplished by pollinating male sterile plants with the heterozygote (*Ms ms*) as the pollen parent. For seedless hybrid production, the seed parent would be a tetraploid inbred.

Once they have been developed, all inbreds can be crossed in all possible combinations. However, that might produce too many entries to evaluate properly. For example, 20 inbreds could produce  $(20 \times 19)/2 = 190$  different hybrids, without including reciprocals. Thus, it may make more sense to make hybrids only from pairs of inbreds having complementing traits of the proper type.

Testing of experimental hybrids should progress in stages, with fewer hybrids to test in later stages where more effort is spent on each hybrid. The first year trials might have two replications in each of two locations. In the second year, the best hybrids could be evaluated in 8 to 12 locations using the conditions available at each (grower fields, state university experiment stations). In the third year, the hybrids would be sent to grower trials throughout the production regions of interest for trials involving 0.25 to 1.0 acre using a total of 5-10 lb. of seeds for all trials. Seeds should be screened for bacterial fruit blotch before sending to growers. One can usually get good data from at least 10 of the 50 trials. Information from the 3 years of trialling should lead to the release of the best one or two hybrids in the fourth year.

Although there is not much advantage of hybrids over open-pollinated cultivars for most traits, it is thought that the former are more uniform. Thus, it may be possible to get the same yield in fewer harvests because of more uniform growth and a more concentrated fruit set. Hybrids offer several advantages over open-pollinated cultivars. A major advantage is the production of seedless triploids, which are produced by crossing a tetraploid female inbred with a diploid male inbred. Hybrids also can express heterosis, with the hybrid performing slightly better than the best parent in some cases. The amount of heterosis in watermelon is around 10%. Another advantage is the ability to get an intermediate fruit shape by crossing an elongate-fruited inbred with a round-fruited one. Inbreds can be used to combine dominant genes for resistance from each parent into a hybrid that has more dominant

genes expressed than either parent. A hybrid that has large seeds for the grower to plant and small seeds in the fruit sold to the consumer can be produced by crossing a large-seeded female inbred with a small-seeded male inbred. Finally, hybrids provide a way for the seed company to protect their proprietary inbreds from theft.

The disadvantages of hybrids are that they add an extra step to the breeding process, and increase the cost of seeds since they are produced by hand pollination rather than by bee pollination. Use of male sterile inbreds for seed production should help reduce the cost of hybrid seeds in the future.

## 7.10 Tetraploid Production

Use of triploid hybrids has provided a method for production of seedless fruit. The tetraploid method for seedless watermelon production was invented by H. Kihara. He began development of tetraploids in 1939, and had commercial triploid hybrids available 12 years later. The development of triploid cultivars adds several problems to the process of watermelon breeding: extra time for the development of tetraploids; additional selection against sterility and fruit abnormalities in tetraploid lines; choice of parents for low incidence of hard seed coats in the hybrids; the reduction in seed yield per acre; reduced seed vigour for the grower; and the necessity for the diploid pollenizer to use up to one-third of the grower's production field.

Seedless cultivars are produced by crossing a tetraploid ( $2n=4x=44$ ) inbred line as the female parent with a diploid ( $2n=2x=22$ ) inbred line as the male parent of the hybrid. The reciprocal cross (diploid female parent) does not produce seeds. The resulting hybrid is a triploid ( $2n=3x=33$ ). Triploid plants have three sets of chromosomes, and three sets cannot be divided evenly during meiosis (the cell division process that produces the gametes). This results in non-functional female and male gametes although the flowers appear normal. Since the triploid hybrid is female sterile, the fruit induced by pollination tend to be seedless. Unfortunately, the triploid has no viable pollen, so it is necessary to plant a diploid cultivar in the production field to provide the pollen that stimulates fruit to form. Usually, one third of the plants in the field are diploid and two thirds are triploid, although successful production has been observed with as little as 20% diploids. Cultivars should be chosen that can be distinguished easily so the seeded diploid fruit can be separated from the seedless triploid fruit for harvesting and marketing.

Breeders interested in the production of seedless triploid hybrids need to develop tetraploid inbred lines to be used as the female parent in a cross with a diploid male parent. One of the major limiting steps in breeding seedless watermelons is the small number of tetraploid inbreds available. Development of seedless hybrids will be discussed in the following stages: (1) choice of diploid lines, (2) production of tetraploid plants, (3) tetraploid line development, and (4) hybrid production and testing.

Stage 1 involves choice of diploid lines to use in tetraploid production. Most of the tetraploid lines being used by the seed industry have gray rind so that, when crossed with a diploid line with striped rind, it will be easy to separate self-pollinated progeny (which will be seeded fruit from the female parent line) from cross-pollinated progeny (which will be seedless fruit from the triploid hybrid). The

grower should discard the gray fruit so they are not marketed as seedless watermelons by mistake.

Stage 2 is the production of tetraploid plants. Many methods have been used effectively in other crops to produce polyploids, including tissue culture regeneration, temperature shock, and X-rays. In watermelon, tetraploids can be produced routinely using plants regenerated from tissue culture or using the herbicide oryzalin. Colchicine ( $C_{22}H_{25}O_6N$ ), a poisonous alkaloid used in the treatment of gout, from the seeds and bulbs of *Colchicum autumnale* is a widely used method in watermelon for tetraploid production. Colchicine inhibits spindle formation, and prevents separation of chromosomes at anaphase. Of all the methods of colchicine application, shoot apex treatment at the seedling stage was found most effective.

For the seedling treatment method, the diploid line of interest is planted in the greenhouse in flats (8x16 cells is a popular size) on heating pads that keep the soil medium at 85°F for rapid and uniform germination. When the cotyledons first emerge from the soil, the growing point is treated with colchicine to stop chromosome division and produce a tetraploid shoot with four sets of chromosomes rather than two. The colchicine solution is used at a concentration of 0.1% for small-seed size cultivars ('Minilee', 'Mickylee', 'Sweet Princess'), 0.15-0.2% for medium-seed size cultivars ('Allsweet', 'Crimson Sweet', 'Peacock Striped', 'Sugar Baby'), and 0.2-0.5% for large-seed size cultivars ('Black Diamond', 'Charleston Gray', 'Congo', 'Dixielee', 'Klondike Striped Blue Ribbon', 'Northern Sweet'). Colchicine is applied to the seedling growing point in the morning and evening for 3 consecutive days, using 1 drop on small- or medium-seed size plants and 2 drops on large-seed size cultivars. The treatment produces plants that are diploid, tetraploid, or aneuploid, so it is necessary to identify and select the tetraploids in later stages. Treatment of the  $T_0$  diploids with colchicine results in about 1% of the seedlings (referred to as  $T_1$  generation tetraploids) being tetraploids. Some diploid cultivars and breeding lines produce a higher percentage of tetraploids than others. For example, 'Early Canada' produces many tetraploids and 'Sweet Princess' does not.

Tetraploids can be detected by the direct method of counting chromosomes of cells under the microscope, or by comparing stem, leaf, flower, and pollen size with diploid controls. A popular method involves counting the number of chloroplasts in stomatal guard cells using a leaf peel under the microscope. Tetraploids have approximately 10-14 chloroplasts in each guard cell (20-28 total on both sides of the stomate), whereas diploids have only 5-6 in each guard cell (10-12 total). The method is useful for screening many plants for ploidy level in the seedling stage before transplanting to the main part of the greenhouse or field nursery for self-pollination. Usually, multiple methods are used, identifying tetraploid seedlings using their phenotype in flats before transplanting, the chloroplast number in the stomatal guard cells of the true leaves in seedling flats and greenhouse pots, and by the appearance of the fruit and seeds at harvest after self-pollination in the greenhouse. Tetraploids usually have thicker leaves, slower growth, and shorter stems than diploids.

Stage 3 involves tetraploid line development. Tetraploid plants are selected (using methods such as leaf guard cell chloroplast number) in the  $T_0$  generation

(plants from colchicine treated diploids) from the greenhouse flats where they were treated with colchicine. It is then necessary to plant the  $T_1$  generation in flats to verify that the plants are tetraploids in that next generation, and transplant the selections to greenhouse pots for self-pollination. Seeds from those selections ( $T_2$ ) can then be increased in larger plantings such as field isolation blocks to get sufficient numbers of seeds per tetraploid line to use in triploid hybrid production.

The fertility and seed yield of tetraploid lines will increase over generations of self- or sib-pollination, probably because plants with chromosome anomalies are eliminated, resulting in a tetraploid line with balanced chromosome number and regular formation of 11 quadrivalents. Seed yield of tetraploid lines in early generations is often only 50-100 seeds per fruit and sometimes as low as 0-5 seeds compared to 200-800 seeds for diploids. Another problem with early generation tetraploids is poor seed germination, making it difficult to establish uniform field plantings. It may require as much as 10 years of self-pollination before sufficient seeds of tetraploid lines can be produced for commercial production of triploid hybrids. Advanced generations of tetraploid lines usually have improved fertility, seed yield, and germination rate compared to the original lines. Some companies require more than 100 lbs. of seed of a tetraploid inbred to be available before beginning commercial production of the triploid hybrid cultivar. Approximately 110 tetraploid plants are required for production of each pound of triploid seeds.

Stage 4 is the evaluation of tetraploids (usually  $T_3$  generation or later) as parents of triploid hybrids. The tetraploids should be evaluated directly for rind pattern, high seed yield, and other traits such as male sterility for reduced hand labour in hybrid seed production. The major test for tetraploids however, is as female parents in triploid hybrid seed production after making controlled crosses using diploid male parents. The resulting hybrids are tested in yield trials with two rows of triploid plots alternating with one row of diploid plots to assure adequate pollen for fruit set in the triploid hybrids. Useful tetraploid inbreds should produce triploid hybrids with excellent yield and quality for the market type and production area of interest.

### 7.11 Triploid Evaluation

Evaluation of triploid hybrids is similar to evaluation of diploid cultivars already discussed. There are a few special considerations, however. Triploids are not inherently superior to diploids, so triploid hybrids can be better or worse than their diploid parental lines. Therefore, as in the case of diploid hybrids, many combinations of parental lines should be evaluated in triploid yield trials to identify the ones producing hybrids with the best performance. In general, diploid inbred parents that have poor horticultural performance will produce triploid hybrids having poor performance.

One problem affecting triploid hybrids is empty seed coats (coloured or white) in the fruit. Under some environmental conditions, fruit are produced with large obvious seed coats that are objectionable to consumers. Triploid fruit should be evaluated for seed coat problems during trialling. Some selection should also be done on the parents before triploid production. Seed coats will be large in the hybrids if the parents have large seeds. Seed size is genetically controlled, with at least three

genes involved: *l*, *s*, and *ts*. Use of tetraploid lines with small or tomato-size seeds may help solve the problem. Besides genetic effects, certain unknown environmental conditions seem to increase the number of hard seed coats in poor performing triploid hybrids.

Commercial production of elite triploid hybrid seed is done by hand in locations where labour is inexpensive, or by bee pollination in isolation blocks. The tetraploid and diploid inbreds are planted together in alternating rows, or in alternating hills within each row. Where labour is abundant, the staminate flowers can be collected from the male (diploid) parent and used to pollinate the pistillate flowers on the female (tetraploid) parent. Pollinated flowers should be capped the previous day to keep bees out, then covered after pollination to prevent self or sib-pollination after the cross has been made. The flowers should be tagged with the date so that the fruit can be harvested 35-50 days later.

A method that requires less hand labour is to plant the pollen and seed parents in alternating rows, and to remove all pistillate flowers from the seed parent rows during flowering time, usually a period lasting several weeks. Pistillate flowers on the female parent are tagged on the day they open with the date to assure that the fruit are mature when harvested, and to harvest only fruit that were pollinated during the time staminate flowers were removed from the female parent. Seeds that are harvested can also be sorted mechanically for size, weight or density to separate triploid seeds (resulting from cross pollination) from tetraploid seeds (resulting from self- and sib-pollination).

When seed production is by bee pollination in isolation blocks, the tetraploid flowers are sib- or cross-pollinated 84% of the time, producing 3x and 4x seeds (progeny). If the 2x and 4x parents of the 3x hybrid have different rind patterns, each of the three-ploidy levels can be distinguished at harvest. For safety, the pollen parent plants should be destroyed after fruit are set on the seed parent plants. A useful combination is for the tetraploid parent to have fruit with a gray rind pattern, and the diploid parent to have fruit with wide stripes, so the resulting triploid hybrid will have striped fruit, easily distinguished from the gray fruited tetraploids that result from self- or sib-pollination of the female parent.

## 7.12 Seed Harvest Mechanization

The job of watermelon breeding can be made easier and more efficient if mechanization is used for as many steps in the process as possible. Small plot equipment can be used for fieldwork to permit more germplasm to be tested with fewer workers and at a lower cost. Small-plot seeders can be used to plant seeds in the field with optimum seed spacing and planting depth using fewer workers than if seeds are planted by hand. If transplants are used to plant the test plots, machine transplanters can be used to punch the hole before the workers on the machine set the seedling into the hole, and follow up with water and fertilizer after the worker has pressed soil around the seedling, all while riding down the field row. Seeds can be packeted using a seed counter, and plot size can be optimized to gain the maximum information for the lowest cost. Research indicates that optimum plot shape is rectangular and block (replication) shape is square. It is difficult to mechanize



harvest since it is done by hand, and each fruit is counted and weighed. However, some efficiency can be gained by using portable computers to collect and analyze data. In the advanced trials, it is useful to estimate flesh sweetness (fruit soluble solids content) using a refractometer, and rind toughness using a spring-loaded punch or penetrometer.

If a greenhouse generation is used to expedite inbred development or hybridization, automation systems are useful for handling the many plants to be grown for self- or cross-pollination. Such systems include automatic heating and cooling, drip irrigation with fertilizer and/or other chemicals injected into the water, trellis support for easy vertical training of the plants, automatic overhead curtains to keep the greenhouse from overheating during the day in the summer, and to keep the greenhouse warmer at night in the winter. Computer systems can provide efficient control of the greenhouse equipment and help provide optimum conditions for plant growth.

For seed harvesting and handling, it is useful to have a bulk seed extractor, washing screens, a seed sluice, and seed dryers. Seed companies have used such machines for years, and it is useful for the plant breeder to build smaller versions that match the size of the plant breeding program. Watermelon breeding is a labour intensive job, but mechanization can help make the most of the available workers, funds, and time.

## **8 Integration of New Biotechnologies into Breeding**

Biotechnologies use an organism or a biological product to manipulate living cells and their molecules. In watermelon, biotechnology is being used to propagate plants in tissue culture, to study genes at the molecular level, to develop molecular markers for selecting useful genes, to isolate the DNA of useful genes, and to incorporate useful genes into cultivars using genetic transformation.

### **8.1 Tissue Culture**

Tissue culture involves the production of cells or plants from plant parts. It offers a method for propagation of valuable plants such as tetraploid parental inbreds, or triploid seedless hybrids. With the increased demand for seedless cultivars, breeders are interested in producing tetraploids in quantities large enough for a hybrid seed production block. Since tetraploid watermelons have slow growth and low seed production, tissue culture can be useful for multiplying new tetraploid lines. Protocols have been developed for propagation of tetraploid plants.

Methods have been developed for the production of tetraploid plants by the regeneration of cotyledons of seedlings cultured *in vitro*. It was possible to produce tetraploid plants from different watermelon cultivars, and was an efficient alternative to the standard method of using colchicines to double the chromosome number of diploid plants.

## 8.2 Marker Assisted Selection

Molecular markers (usually sequences of DNA) can be used as reference points in mapping genes on a chromosome. The information is useful in the selection of plants that carry a marked gene of interest. Plant breeders can discard plants from a segregating population that are missing the gene of interest. In that way, field testing can be done using only the plants having a particular set of traits. Markers can also be used to identify cultivars (DNA fingerprinting), and to estimate the genetic relatedness of a set of cultivars or individuals in a population.

Over 40 genes have been described in watermelon. The genes are involved in disease resistance, flower type, fruit shape, and fruit quality. If molecular markers can be identified that are closely linked to those genes, then selection might be performed more rapidly, or earlier. For example, selection might be carried out in a seedling test instead of waiting for the plants to produce fruit.

Molecular markers have been used to estimate genetic relatedness of watermelon cultivars, and can be used to evaluate inbred lines for purity. More than 60 DNA sequences have been published, and some have been used to construct linkage maps. Finally, DNA markers have been used to detect the presence of pathogens on watermelon seeds.

## 8.3. Genetic Transformation

Genetic transformation provides methods for inserting single genes into plants while overcoming barriers to interspecific crossing. The soilborne bacterium *Agrobacterium tumefaciens* is often used as the vehicle for transferring genes into plants. Plant cells can also be transformed using microparticle bombardment, where DNA-coated particles are shot into plant cells. Several studies have reported transformation of watermelon using *Agrobacterium* or microparticle bombardment. Transformed cells must be regenerated from sterile culture to produce plants containing the new gene to be used in a breeding program.

Transformation of watermelon plants has been used to confer virus resistance. Several virus species cause disease in watermelon. These include Squash mosaic virus (SQMV), Cucumber mosaic virus (CMV), Papaya ringspot virus-watermelon strain (PRSV-W), Zucchini yellow mosaic virus (ZYMV), and Watermelon mosaic virus (WMV). Transfer of virus coat protein genes into watermelon plants may confer resistance to the virus disease, and provide plant breeders with new resistance genes.

## 9 Seed Production

Early watermelon cultivars were mostly inbred lines produced commercially by open pollination of bulk-increased or hand-pollinated breeder seeds. In the 1970s, large-scale production of diploid hybrid seed began. Diploid hybrids have now taken over most of the commercial production in North America, Western Europe, and Japan.

## 9.1 Hybrid Production

Hybrid seeds are produced in the seed parent by pollination from staminate flowers in the male parent. Hybrid production can be by hand pollination using inbred lines grown in adjacent rows in the field, or by planting the two parental lines in an insect-proof cage. Pollinations are marked for later seed harvest using tags or bags after pollination. Each fruit will have 200 to 800 seeds, and fewer than 4000 seeds are needed per acre of commercial production.

A less expensive alternative to hand pollination is to plant the two parental inbreds in an isolation block. Staminate flowers are then removed daily from the plants in the seed parent rows to avoid self- and sib-pollination. All pistillate flowers in the seed parent row that are pollinated during the days of staminate flower removal are tagged for hybrid seed harvest. Another solution would be to incorporate a recessive seedling marker such as non-lobed leaf or the glabrous gene into the seed parent. Seedlings resulting from self- or sib-pollinations would have the marker and could be removed from the planted field or removed from the transplant flats to get 100% hybrid seedlings. Conversion of the seed parent to a near-isogenic male sterile line offers the possibility of hybrid seed production without the work associated with the above three methods. However, genetic male sterility requires that male fertile plants be rogued out of the seed parent rows in the hybrid production block.

Seeds can be sorted after the seed cleaning operation by size, weight, or density to increase the proportion of hybrid seed in the lot. Diploid open-pollinated seed yields should be higher than 251 lb./acre (average for United States in 1976-1977). Very good seed yields would be 400 lbs./acre. Triploid seed yields average about 20-40 lbs./acre (about 10% what diploids would produce).

## 9.2 Commercial Systems

Most commercial watermelon seed production is located in arid or semi-arid areas of the world such as western China, Chile, Mexico, Thailand, and the United States (California and Colorado). Arid conditions favour the production of high quality, disease-free seeds. With the outbreak of bacterial fruit blotch of watermelon in the late 1980s, seed production in areas of low humidity and no rainfall has become even more desirable in order to produce disease-free seed.

Sanitation is important at all stages of production. Workers should wash their hands with antibacterial soap or rinse them with 70% isopropyl alcohol before handling plants or fruit and between seed lots. All equipment should be cleaned and all soil and plant material removed before use in production areas. Clean and disinfect harvesting tools and equipment with alcohol or 0.5% NaOCl or Ca(OCl)<sub>2</sub> between seed lots. Sanitation, harvest, and control procedures for production of foundation and stock (parent) seed should be at least as stringent as that for commercial seed.

The process of growing watermelon seed crops is similar to that for growing market crops except that site selection is more critical. Choose a field that has not had any cucurbits (watermelon, cantaloupe, honeydew, cucumber, summer or winter squash, pumpkin, or gourd) in it for at least 2, but preferably 4 years. A field that has

a history of *Fusarium* wilt or anthracnose should be avoided. Fields for open-pollinated watermelon seed production should be isolated by at least 1 mile from other watermelon fields to prevent contamination by outcrossing. Isolation also prevents disease spread from fields containing watermelon and cantaloupe crops of unknown origin or planted with seeds that have not been tested for seed-borne disease. The production site should be as far as possible from fields where bacterial fruit blotch occurred the previous year to reduce contamination from leftover debris. Wild cucurbits, such as citron and volunteer watermelons, must be removed from a 1-mile radius surrounding the production field to eliminate outcrossing and disease contamination.

Selection of parental seed from elite or foundation seed is the first critical element of seed production. Use seed that was produced in dry climates and has been tested to be free of the pathogens causing gummy stem blight, watermelon fruit blotch, anthracnose, and squash mosaic. Direct-seeded plantings reduce the risk of seedling contamination in greenhouses. If transplants are used, they should be produced in a greenhouse that does not contain other cucurbits. Irrigation of transplants in the greenhouse preferably should be from an ebb and flow or a float system. Overhead irrigation of seedlings in the greenhouse should be avoided. Greenhouses for transplant production should have good air circulation and low relative humidity.

Drip or furrow irrigation should be used in the production field instead of overhead irrigation to reduce leaf wetting and disease spread. Roguing of off-type and diseased plants within the field should be done throughout the growing season. There are four useful stages for roguing. The first is before flowering when vegetative characters are checked. The second stage is at early flowering when morphology of undeveloped fruit is checked. The third stage is when the developing fruit are checked for trueness to type, and the final roguing is confirming the external morphological characters of the fruit to be harvested. Roguing for off-types is not effective after pollination in a field for open-pollinated seed production. It is only effective when fruit have been self or cross-pollinated and the male has no off-types. Inspectors should be trained to recognize variations in watermelon fruit blotch symptoms.

Preventative applications of copper fungicide can also help in reducing fruit blotch contamination of seed. The first spray should be 2 weeks before flowering. Application of registered fungicides will reduce gummy stem blight seed contamination. Seed should not be harvested from fields where there is confirmation of fruit blotch or until the possibility of fruit blotch is eliminated. Seeds harvested from fields in which fruit blotch is confirmed or which were adjacent to contaminated fields should not be used.

All fruit should be inspected by trained technicians for symptoms that are suspected to be fruit blotch. All fruit suspected of having fruit blotch must be discarded. No fruit should be harvested from vines that have anthracnose or gummy stem blight symptoms. When seeds of open-pollinated fruit, and in some cases, hybrid fruit, are mature the fruit are windrowed by machine. Windrowed fruit are picked up by self-propelled vine seed harvesters that crush the fruit and separate the seeds and pulp from the rind. For some hybrid seed production, fruit are harvested by

hand and various sized seed extractors are used. In either case, the diploid seed slurry is transferred to bins where it is allowed to ferment for 24 to 48 hours. During this time the sugars and gelatinous material surrounding the seeds are degraded.

Fermentation plus acid washing (1% hydrochloric acid) can reduce the chance of seed transmission of fruit blotch. Fermentation and acid treatment of triploid seed reduces seed viability, so is not recommended. Seeds extracted from tetraploid fruit for triploid seed production should be washed immediately. Seeds are separated from pulp and juice by washing in a rotary washer or flume system. Some seed lots are dried by heat from the sun. However, higher quality seeds are produced using forced air warmed by propane heaters. Seeds are placed on flat drying beds or in large rotary dryers. Dry seeds are run through a mill containing sizing screens that separates large seeds from trash and small seeds.

All seed lots should be assayed for the presence of the fruit blotch bacterium, squash mosaic virus, and gummy stem blight pathogen by the best methods available. In Asia, cucumber green mottle virus is a problem and is seed transmitted. For fruit blotch, seedling grow-outs of at least 10,000 seeds per lot are currently used, but polymerase chain reaction (PCR) techniques may provide more efficient and sensitive methods. Coupling seedling grow-outs with PCR may be necessary for some situations. Squash mosaic virus can be screened with grow-outs. For gummy stem blight, seedling grow-outs or blotter tests using a minimum of 1,000 seeds per lot are recommended. However, PCR techniques may provide better methods in the future. Commercial seeds should be treated with a registered protectant such as Captan and Thiram before sealing them into cans, bags, or packets. Seeds should be stored in hermetically sealed containers at 6.5% (no greater than 10%) moisture content. Under favourable storage conditions, seeds should last 4 years. To be salable, germination of the seed lot must be at least 70%.

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